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Nerve Degeneration Associated With Avitaminosis A in the White Rat

T. S. Sutton, H. E. Setterfield, and W. E. Krauss

OHIO
AGRICULTURAL EXPERIMENT STATION
Wooster, Ohio

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T. S. SUTTON, H. E. SETTERFIELD[1], AND W. E. KRAUSS

INTRODUCTION

NOTES ON THE DEVELOPMENT OF THE PRESENT CONCEPT OF VITAMIN A IN NUTRITION

Studies of the effects of nutritional deficiencies on the tissues of the animal body now constitute an important field of research. Such studies are fundamental in establishing a conception of an adequate diet. The dieting of an animal on the borderline of deficiency may result in changes in structure and function which are so stealthy in their onset that irreparable damage is done before external symptoms are noted. Furthermore, the maintenance of an efficient defense mechanism against invading microörganisms is in a measure under dietary control. An adequate diet must do more than supply the necessary factors for life, growth, and body activity; it must adequately supply the necessary factors for the maintenance of health as well.

Rickets is a disease which might be used to illustrate this point. This disease involves tissue changes which present no external manifestations, at least in the early stages. The animal continues to grow; in fact, it is difficult to produce rickets experimentally unless the animal does grow. However, an examination of the bony structures of the body proves beyond doubt that tissue changes have occurred.

The successive steps in the development of the present concept of vitamin A in nutrition have occurred in quite a different manner from those of most of the other vitamins. The discovery of some of the vitamins, notably B, C, and G, developed primarily from a study of diseases produced by their deficiency in the diet. Beri-beri and scurvy were recognized as nutritional diseases long before the explanation of their preventative or curative treatment was revealed. The same might be said of pellagra; however, the curative factor in this case was so closely associated with the anti-beri-beri vitamin that for some time its nature was obscured. On the other hand, vitamin A was discovered as a result of the nutritive failure of animals on purified diets, quite aside from a study of a particular disease.

Hopkins (17) pointed the way to the discovery of new dietary essentials when he employed diets of purified proteins, carbohydrates, fats, and salts in animal experimentation. The early attempts to nourish experimental animals on these purified diets resulted in baffling failures. A few years later he (18) found that, by adding small amounts of alcohol-soluble substance or substances found in milk, the inadequacy of his purified diets could be overcome. Furthermore, he produced evidence that the material added was not any of the previously known constituents of milk.

[1]Anatomy Department, College of Medicine, The Ohio State University.

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In the light of our present knowledge it is thought that this alcohol-soluble material prepared by Hopkins contained both water-soluble and fat-soluble factors. Although this fact was not recognized by Hopkins, it is now known that these factors are fairly soluble in alcohol. This probably accounts for the nutritional success obtained when this alcohol extract was added to the diet of purified, known dietary essentials.

It remained for McCollum and Davis (28) and Osborne and Mendel (40), working independently, to show that the fat of milk contained a growth-promoting substance. This growth-promoting property possessed by the fat of milk could not be attributed to the triglycerides but rather to some material dissolved in the fat and contained in the non-saponifiable fraction. That such a material existed had been demonstrated several years earlier by Steppe (46). Although Steppe demonstrated that some substance associated with the alcohol-ether extract of foods which was destroyed by prolonged heating was necessary for normal nutrition, he failed to recognize the nature of the substance. He believed, however, that some of the fat-like compounds such as lecithin, cholesterol, and cephalin were indispensable dietary constituents.

With regard to the eye condition which later was shown to be caused by a lack of vitamin A in the diet, a comparable condition was described by Knapp (24) in 1908. In fact, in commenting on his observations, he almost postulated a specific prophylactic substance such as that of a fat-soluble vitamin when he wrote, "Es nicht nur die prämortale Resistenzlosigkeit war, welche die Tiere zu derartigen Katarrhen pradisponirte, sondern, wir eine weitere Ursache in der Art der Nahrung suchen müssen, vielleicht in dem Fehlen eines ganz bestimmten wichtigen Körpers."[2]

In his earlier studies McCollum unknowingly supplied the water-soluble factor in the lactose of his purified diets. Consequently, he believed that the fat-soluble factor was the only hitherto unidentified material necessary for normal nutrition. Later he recognized the necessity of both water-soluble and fat-soluble substances. In order to avoid objections of nomenclature experienced by Funk with his term "vitamine" and Hopkins with his "accessory factor" McCollum and Kennedy (29) suggested that these dietary essentials be known as "fat-soluble A" and "water-soluble B".

The present naming system dates back to 1920 when Drummond (4) suggested that the designations of Funk and McCollum be combined. He suggested that the final "e" of Funk's "vitamine" be dropped so that the term would no longer be suggestive of the chemical nature of the substance. The resulting word "vitamin" was then to be used with the familiar alphabetical designation. Thus, we have vitamin A, vitamin B, vitamin C, etc.

Prior to 1922 both anti-ophthalmic and anti-rachitic properties were attributed to vitamin A. However, experimental data appeared in the literature before this date, which, in the light of our present knowledge, could be interpreted as demonstrating the existence of two fat-soluble vitamins. Final proof of the existence of two fat-soluble factors was provided by McCollum and co-workers (32). By oxidizing cod-liver oil with air at 100° C. for 12 to 20 hours the vitamin A was destroyed. This oxidized oil would cure rickets but not ophthalmia, thereby demonstrating that the anti-rachitic factor was not vitamin A. These findings were later confirmed by Steenbock and Nelson (45).

[2]Translated. It was not alone the premortal loss of resistance which predisposed these animals to these catarrhal inflammations but an additional cause must be sought for in the diet, perhaps in the lack of an important element.

Reports of the experimental work dealing with the effects of vitamin A deficiency on the animal body which appeared in the literature prior to 1930 have been reviewed and summarized by McCollum and Simmonds (31) and Sherman and Smith (44). When diets lacking or low in vitamin A are fed to young animals, they continue to grow for a while at an almost normal rate. The length of this period of almost normal growth depends upon the store of vitamin A in the body at the beginning. After this stored supply of vitamin A is exhausted the growth rate diminishes and soon comes to an almost complete stop. After growth cessation a period of general decline follows with rapid loss of weight leading to death.

Almost concurrent with cessation of growth an eye disorder develops in a large percentage of the cases. The pathology of this eye condition has been studied by several investigators who are largely in agreement on their findings. There is a xerosis of the epithelial tissues of the cornea and conjunctiva. The outer epithelial cells become flattened and are loosened from the cell layers underneath. This outer layer of xerotic cells is shed by a process of desquamation. The defense mechanism against microörganisms is weakened, and infections result from the invasion of ubiquitous organisms.

These findings led to the investigation of epithelial tissues elsewhere in the body. Marked changes have been found in the paraorbital glands, the salivary, and accessory salivary glands. There are xerotic changes in the epithelium of the respiratory tract down to the bronchioles. In the alimentary tract changes have been noted in the epithelium of the mouth, oesophagus, and cardiac stomach. No epithelial changes have been reported in the remainder of the stomach or in the large or small intestines. The epithelial lining of the bladder, ureters, and pelvis of the kidney have been found to be affected. It is now generally accepted that the lack of vitamin A alone is sufficient to induce metaplasia of columnar, cuboidal, and transitional epithelia to the squamous keratinizing type in some organs.

The cause of reproductive failure among animals on vitamin A-deficient diets has been studied. The preponderant evidence points to atrophy of the germinal epithelium of the testicle of the male and ovarian disfunction in the female. In the female the pathology is particularly significant. The follicles develop but are unable to rupture. This results in a prolonged or persistent presence of cornified epithelium in the vagina. Since this is one of the earliest signs of vitamin A depletion, the examination of vaginal smears has been suggested as an accurate method for early diagnosis of vitamin A deficiency.

The comprehensive reviews by McCollum and Simmonds (31) and Sherman and Smith (44) contain no mention of nervous disorders associated with vitamin A avitaminosis.

Early in 1931 we noted symptoms indicative of nervous disorders in certain groups of rats used for vitamin A assays. The highest incidence of the symptoms in question was among groups receiving the lower levels of vitamin A supplement. Extreme ophthalmia, emaciation, and weakness with subsequent early death frequently masked the symptoms in negative controls. These rats from dams receiving our regular stock diet were weaned when 24 days old and depleted of vitamin A according to our usual procedure. After 40 to 50 days on the vitamin A-free diet, followed by low levels of vitamin A after depletion, these animals developed symptoms which had not previously been described as associated with avitaminosis A in the white rat.

The data in the literature at that time dealing with possible similar conditions were so meager and conflicting that it was thought desirable to attempt to obtain further information that might help to clarify the situation.

Krauss and Hayden (25) briefly described these symptoms. Over 50 per cent of the rats receiving vitamin A-deficient diets showed paresis of the rear limbs. These rats retained the impaired condition after sufficient vitamin A was added to the diet to promote growth and to cure ophthalmia.

The atrophy of the musculature associated with this disorder suggests the importance of its consideration in the present technique of biological assay for vitamin A. It follows that a study of the etiology and pathology of the disease is essential before any studies of its effect on the results of biological assays for vitamin A are undertaken.

REVIEW OF THE LITERATURE

Hart and co-workers (15) demonstrated degeneration of the motor cells in the spinal cords of swine exhibiting nervous symptoms induced by rations high in wheat meal or wheat middlings. Although the rations which they employed were low in both vitamin A and inorganic elements, they ascribed the nutritive failure to a toxicity of the wheat germ. This condition was best alleviated by the addition of alfalfa meal or meat scraps to the diet. These materials exerted a preventative action even though the relative amount of wheat germ in the ration was not changed.

Wolbach and Howe (50) in an exhaustive study of tissue changes in vitamin A-deficient rats found no degenerative lesions in the brain or sympathetic ganglia. The ganglion cells of the mesenteric plexus were normal whenever found. Their animals showed no symptoms which were indicative of nervous disorders. An investigation of the peripheral nerves was not included in this study. The diet employed by Wolbach and Howe was admittedly low in vitamins C and D, as well as in A. Goldblatt and Benischeck (14), using a diet lacking only in vitamin A, confirmed the work of Wolbach and Howe as far as tissues other than nerves were concerned. This at least indicates that these earlier workers were dealing with vitamin A deficiency. Goldblatt and Benischeck did not study the nervous system.

Kingery and Kingery (23) reported degeneration in the central and peripheral nervous system of rats on a diet deficient in vitamins A and B. The Marchi technique of fixing and staining was employed.

Mellanby (33) reported that, when wheat germ was added to the diet of dogs to the extent of 10 per cent of the cereal, severe nervous symptoms developed. He ascribed the action of this diet to toxic substances in the wheat germ. He believed that these toxic substances were counteracted or rendered innocuous by fat-soluble vitamins. He was able to demonstrate degenerative changes in the spinal cords fixed and stained after the method of Marchi.

He extended his investigations (34) and added further proof that the lack of vitamin A was a causative factor in producing these symptoms. He described the symptoms as a spasticity which may be masked by weakness. The hind legs were more frequently affected than other parts of the body. Convulsions were frequent. The changes occurring in the nervous system were the same as he had noted in 1926.

In this study Mellanby again attributed the disorders to a positive harmful influence of wheat germ, rye germ, or ergot in the absence of a defending chemical mechanism (vitamin A or carotene). The administration of rich sources of vitamin A or carotene (carotene, carrots, egg yolk, cod-liver oil, butterfat, or cabbage) prevented the degeneration or caused improvement in the paralytic symptoms after they had once developed, the degree of improvement depending upon the extent of the initial involvement.

In examining the data presented by Mellanby, it seems significant that no normal spinal cords were encountered in dogs which received a vitamin A-deficient diet. Control diets which did not contain wheat germ, rye germ, or ergot were used in three of the experiments reported, and some degeneration was noted in each case. Furthermore, these animals were not normal in their movements. It was only in those animals which received diets adequately supplemented with vitamin A that no degeneration was reported, and these were the only animals which exhibited a normal gait.

White corn as the cereal ingredient of the diet was not protective against nerve degeneration. On the other hand, yellow corn was protective even when 2 grams of ergot were included in the ration. Butterfat which had been oxidized for 12 hours was impotent as a protective agent.

Mellanby (35) reported a further extension of his investigations along this line in 1933. He found myelin degeneration in the peripheral nerves, as well as in the spinal cord, of dogs on a diet of separated milk, cereal, lean meat, a vegetable fat, yeast, and orange juice. These degenerative changes could not only be prevented by including in the diet a rich source of vitamin A or carotene, but they usually failed to occurr when all the cereal of the diet was replaced by potato, even though the diet was deficient in vitamin A. This observation leads to confusion in interpreting the cause of the nerve degeneration but fits in well with Mellanby's theory of cereal toxicity.

Hughes and co-workers (19, 20) noted symptoms of nervous disorders in pigs kept on a diet consisting of white corn 87 per cent, tankage 10 per cent, and bone ash 3 per cent for about 13 months. The symptoms were characterized by impaired vision, incoordination of movements, and spasms or convulsions. There was no complete paralysis of parts. An examination of parts of the nervous system after the method of Marchi showed degeneration in the optic thalamus, in the optic, femoral, and sciatic nerves, and in certain areas of the spinal cord. These disorders were prevented by including cod-liver oil, butterfat, yellow corn, or alfalfa leaf meal in the diet. These workers also mention that rats in the last stages of vitamin A deficiency showed a decided unsteadiness of gait which seemed to be caused by some impairment of the nervous system.

Duncan (5), in studying the incidence of secondary (Wallerian) degeneration in normal mammals compared to that in certain experimental and diseased conditions, found that the rate of degeneration in the sciatic nerves of rats was increased in vitamin A deficiency. In normal animals (animals receiving the basal diet plus yeast and cod-liver oil) the rate of degeneration increased with age. This degeneration rate was still further increased approximately three and one-half times by feeding a diet low in vitamin A; that is, the number of degenerating fibers (sciatic nerve) was approximately three and one-half times as great in the A-deficient animals as in the corresponding control animals. The highest number of degenerating fibers was found in a vitamin A-deficient rat which was free from infections. This indicates that the nerve degeneration is not caused by toxins resulting from bacterial invasion.

Suzmann and co-workers (48) attempted to produce spinal cord degeneration in dogs fed a high cereal diet (rolled oats 76.8, sugar 12.8, lard 6.4, bone ash 2.4, and salts 1.6). These workers failed to demonstrate any neurological changes but report a syndrome of anemia, skin lesions, anorexia, and change in the blood lipids.

Elvehjem and Neu (8) reported a muscular incoordination in chicks affected by avitaminosis A. These workers suggest the possibility of these symptoms being due to nervous degeneration.

Zimmerman (51) observed incoordination and spastic paresis in animals on diets low in vitamin A. The diets consisted of casein, corn starch, Crisco, salts, yeast, and irradiated ergosterol. The symptoms observed led to a macroscopic and microscopic examination of the nervous system. Seven groups, comprising a total of 23 animals, were used. The conditions varied in the different groups with respect to the diets of the dams during pregnancy and suckling and the stage of deficiency at which the animals were sacrificed. Parts of the nervous system were prepared for examination according to Marchi, Spielmeyer, and Scharlach R techniques.

No gross abnormalities were found in any part of the nervous system. A microscopic examination revealed degenerative changes in the myelin sheath of the brachial plexuses, sciatics, and vagus nerves. In the spinal cord the sensory tracts were most extensively involved. Only one animal showed a microscopical abnormality of the brain.

The degenerative changes did not precede the clinical symptoms of degeneration by any appreciable period. For a short but undetermined period after recovery marked lesions were still present. One rat which had shown the clinical symptoms for 43 days did not completely recover in 29 days of cod-liver oil feeding; however, definite improvement was noted.

Attention is called to the fact that the diet employed contained no cereals which might have contributed a toxic substance and that a deficiency in fatty acids was not probable. Description of the symptoms, together with details of the dietary conditions and the time of development of the symptoms in this study, is reported by Aberle (1).

Nerve lesions and symptoms indicative of nerve lesions have been noted among animals suffering from other nutritional deficiencies. Of particular note is the deficiency in the vitamin B complex. Accurate information concerning the exact cause and nature of these disorders is extremely limited. The reasons for such a state of affairs are obvious: In the first place, what was formerly thought to be a single factor has now been resolved into four components—B or B_1, the fraction which prevents polyneuritis in fowls, a deficiency disease first produced experimentally by Eijkmann (7); B_2 or G, the anti-pellagra factor [Goldberger and Tanner (12)]; B_3, the thermolabile pigeon factor of Williams and Waterman (49); and B_4, a thermolabile water-soluble accessory factor necessary for the nutrition of the rat [Reader (43)]. Secondly, the discovery of the factors B_3 and B_4 has been so recent that no great amount of work has been done on tissue changes produced by their deficiency in the diet. A third disturbing factor is the report of toxic substances isolated from a number of foodstuffs which produce pellagra-like symptoms and tissue changes [Stockman and Johnston (47)]. These toxic substances seem to be similar in their physiological effect to Mellanby's cereal toxins.

Conflicting evidence has been produced in some instances, probably because of improper control of diet variables. Thus, Moore, Plymate, and Andrews (37) found no degenerative changes in the peripheral nerves of 471 rats on stock and 18 synthetic B-deficient diets. On the other hand, Zimmerman and Burack (52) described nerve lesions of the peripheral nerves of rats receiving a diet deficient in the vitamin B complex. The nervous symptoms reported were similar to those described in B_4 deficiency by Reader (43).

In other instances differences in the techniques employed in fixing and staining nervous tissue have resulted in conflicting results. The Marchi and other osmic acid techniques have been extensively employed in studies of myelin degeneration. Prickett (42), using osmic acid and Sudan III as stains, studied the peripheral and central nerves of rats on diets deficient in vitamin B_1. In the peripheral nerves, particularly in the sciatics, the osmic acid preparations showed darkened areas indicative of degeneration. Material from the same animals stained with Sudan III showed normal nerves in every instance. Studies of this nature reveal the inadequacy of some of the histological and pathological techniques. Inasmuch as Prickett found disseminated foci of hemorrhage and areas of cellular damage in the brain, he concluded that the site of nervous lesions in B_1 deficiency is in the central nervous system.

Keenan and co-workers (22) report a new nutritional factor required by the chick, which they believe to be identical with Reader's B_4. The absence of this factor from the diet leads to nervous disorders and caseous lesions in the cerebrum and cerebellum similar to those described by Pappenheimer and Goettsch (41).

McCarrison's (27) observation that in polyneuritis the nervous tissue is not greatly involved in degenerative changes is generally accepted today. The rapid recovery (within a few hours) upon administration of large dosages of vitamin B is almost sufficient evidence to prove this concept.

Pellagra is primarily a skin and nervous disease. The similarity between the nervous symptoms and nervous lesions of pellagra and those produced by a high-cereal diet in the absence of vitamin A by Mellanby (34) and those produced by a high-cereal diet adequately supplemented with vitamin A by Stockman and Johnston (47) is confusing.

Before we can arrive at any satisfactory explanation of these conditions it seems necessary that a comprehensive comparative study of the nutritional production and the pathology of these deficiency diseases be conducted in a single laboratory by the same group of workers. Such a study is now being contemplated in the laboratories from which this paper originates. It is hoped that the data presented in this communication may be of value in clarifying the situation as far as vitamin A is concerned.

EXPERIMENTAL

EARLY OBSERVATIONS

Early in 1931 we were using quite a large number of rats for vitamin A assays. These rats were raised from our stock colony of a closely inbred Wistar strain of *Mus norvegicus albinus*. The stock colony is maintained on Diet S_6 (Table 1). The litters of young rats were reduced to six (three males and three females) when 3 days of age and weaned when 24 days of age. All the rats used in this study were raised according to this procedure.

When weaned, the rats were placed in hardware-cloth cages (three to a cage) with raised hardware-cloth bottoms until depleted of vitamin A. At the time this study was begun we were using $-A_9$ as a depletion diet. During the study this diet was varied as indicated in Table 1, $-A_{10}$ and $-A_{11}$. Vitamin D was supplied as irradiated yeast or viosterol; vitamins B and G were obtained from yeast. The yeast was a dehydrated powdered product obtained from the Northwestern Yeast Company. The vitamin supplements were fed daily in a separate container. After depletion the vitamin A supplement was fed daily with the yeast.

At the time of cessation of growth (generally between the fifth and sixth week on the A-free diet) about 60 per cent of the animals showed signs of incipient ophthalmia. At this time the vitamin A supplement was added to the daily ration. The rats were placed in individual cages and divided into groups for the feeding of graded amounts of the vitamin A supplement.

TABLE 1.—The Basal Diets Used in This Study

S_6 (the diet on which the stock colony is maintained); $-A_9$, $-A_{10}$, and $-A_{11}$ (the vitamin A-depletion basal diets)

Stock diet S_6		**Deficient diet—A_9**	
Yellow corn meal	67	Corn starch	66
Casein	16	Purified casein	18
Linseed oil meal (O. P.)	12	Crisco or Primex	10
Alfalfa meal	3	Salt mixture (McCollum's 185)	4
$CaCO_3$	1	Agar (coarse powder)	2
NaCl	1	Distilled water	
Whole milk *ad lib.*			
Tap water			
Deficient diet—A_{10}		**Deficient diet—A_{11}**	
Dextrin	66	Dextrin	31
Purified casein	18	Corn starch	31
Lard	10	Purified casein	20
Salt mixture (McCollum's 185) (30)	4	Lard	6
Agar (coarse powder)	2	Crisco	6
Distilled water		Salt mixture (McCollum's 185)	4
		Agar (coarse powder)	2
		Distilled water	

After about 2 weeks on the vitamin A-supplemented diet, symptoms indicative of nervous disorders were noted in certain groups, particularly those receiving less than unit dosages of vitamin A. By this time any negative controls which were still living were so emaciated and weak that any symptoms of nervous disorders which might exist could easily escape notice.

The early symptoms are characterized by a peculiar unsteady, weaving gait in the rear limbs. There is a definite lack of coordination rather than an actual paralysis in the early stages. Frequently, when the rats are moving about, the limb or limbs involved are abducted to a greater than normal degree. The condition is usually unilateral, and in cases of bilateral involvement the symptoms are more pronounced in one limb. With the progress of the symptoms the ability to use the affected parts diminishes. The animal walks back on the heel with little use of the digits. The muscles of the thigh and leg are flaccid, and the digits are usually flexed. We have never noted a spastic or convulsive condition. In this respect our observations differ from those of other investigators in their description of the symptoms of nerve degeneration in vitamin A deficiency. When the rat is moving about, the limb is frequently abducted to such a degree that the animal walks on the side of the heel rather than on the sole of the foot. When the animal is stimulated by pinching the tail or toes of the rear foot, only a feeble response is noted. The final result is an irreparable atrophy of the muscles of the thigh and leg.

Figure I shows an animal in the later stages of the nervous symptoms. This animal had received less than a unit dose of vitamin A (30 mg. of butterfat) for 4 weeks following the depletion period. The eye condition had improved somewhat. The abduction of the limb, the flexed digits, and the apparent, flaccid condition of the musculature of the thigh and leg can be noted in this photograph.

Muscle atrophy has not been previously described as a final result of nerve degeneration in vitamin A avitaminosis. This almost invariably happens in the late stages where life is sufficiently prolonged by a low level of vitamin A feeding after depletion. Where only one leg is involved the atrophy can easily be detected grossly by palpation. Figure IIIa is a photomicrograph of a histological section of a normal biceps femoris from a rat which had received a complete diet. Figure IIIb is a photomicrograph of a histological section from an atrophied biceps femoris. The rat from which this muscle was taken was in about the same physical condition as the one shown in Figure I.

Fig. I.—This is a photograph of rat C701 ♂ showing typical symptoms of nerve degeneration associated with avitaminosis A. Note the abducted limb, the flexed digits, and the apparent, flaccid musculature of the thigh and leg. This rat received 30 mg. of butterfat daily for 28 days following depletion.

PRELIMINARY STUDIES

Irradiated yeast had always been used as a source of vitamin D in the depletion diet for vitamin A assays in this laboratory. Yeast to be used for this purpose was spread out in a thin layer and irradiated for 20 minutes with a Cooper-Hewitt quartz mercury lamp at a distance of 20 inches. Yeast treated in this manner had not been assayed for vitamin D in this laboratory. Therefore, we decided to compare the occurrence of nervous symptoms among animals receiving irradiated yeast and animals receiving a known quantity of viosterol which had been previously assayed for vitamin D. A group of 17 rats was dieted according to our usual procedure for vitamin A depletion, with the exception of a vitamin D supplement. One division of nine animals received the usual 0.4 gram of irradiated yeast, while the remaining eight were given the same amount of yeast (not irradiated) and approximately 2.6 units of vitamin D in the form of viosterol daily.

In comparing the two groups no significant difference in the development of ophthalmia or nervous symptoms was noted (Table 2). The rats receiving the irradiated yeast developed ophthalmia after 37.2 days (average) and nervous symptoms after 52.5 days (average) on the A-free diet. Those receiving viosterol as the source of D developed ophthalmia after 37.8 days (average) and nervous symptoms after 50 days (average) on the A-free diet. Inasmuch as no significant difference in the development of nervous symptoms was noted, we concluded that a lack of vitamin D in the irradiated yeast could not be responsible for the development of nervous symptoms among the rats used for vitamin A assays.

TABLE 2.—The Development of Ophthalmia and Nervous Symptoms in Rats on Diet —A_9 with Different Vitamin D Supplements

Rat No.	Days on deficient diet before ophthalmia developed	Days on deficient diet before nervous symptoms developed
Diet—A_9 + 0.4 gm. irradiated yeast daily		
C 319 ♀	32	Died before symptoms developed
C 314 ♀	33	49
C 318 ♂	33	45
C 316 ♀	35	Died before symptoms developed
C 320 ♀	36	58
C 312 ♀	41	55
C 313 ♂	41	58
C 315 ♂	41	46
C 317 ♂	43	57
Diet—A_9 + 0.4 gm. unirradiated yeast + approximately 2.6 units vitamin D (viosterol) daily		
C 322 ♂	32	56
C 323 ♂	32	Died before symptoms developed
C 321 ♀	33	49
C 324 ♂	41	44
C 327 ♂	41	51
C 328 ♀	41	46
C 329 ♀	41	50
C 326 ♀	42	54

We had noted that some of the rats in advanced stages of avitaminosis A showed external symptoms indicative of anemia. Since it is known that there is nerve degeneration in anemia [Boyd (2)], a study of the relationship of the blood picture to the nervous symptoms was attempted in a second preliminary study (Table 3). At the suggestion of the late Dr. F. L. Landacre, who had worked with Evans of California and was familiar with the nervous symptoms associated with vitamin E deficiency (10), part of the animals in this group was given 50 mg. of wheat germ oil daily. Others received drinking water containing 0.1 per cent of copper sulfate. It will be noted that the depletion period in this group of rats was somewhat longer than that of the previous group. This can be accounted for on the basis of a greater body store of vitamin A at the time of weaning. The mothers of these rats received milk from pasture-fed cows during pregnancy and lactation, while those in the previous group received milk from cows on winter feed. We have found that under our usual feeding conditions the butterfat from pasture-fed cows has about 2.5 times the vitamin A activity of the butterfat from cows on winter feed.

The hemoglobin and erythrocyte determinations reported in Table 3 were made the same day that the animals were killed unless otherwise specified. Hemoglobin determinations were made with a Dare hemoglobinometer, and erythrocytes were counted in a Hellige counting chamber with improved Neubauer double ruling. Somewhat higher than normal values for hemoglobin and erythrocytes indicate that anemia is not a factor in the development of the nervous symptoms. The addition of 0.1 per cent of copper sulfate to the drinking water produced no significant difference in the blood picture. Our observation of an increase in red blood cells in avitaminosis A is in agreement with the findings of Falconer and Peachy (11). They suggest that the increase may be due to a diminution of the blood volume. The paleness which we observed may have been caused by a disturbance of the vaso-motor mechanism.

The inclusion of 50 mg. of wheat germ oil in the ration did not prevent or retard the development of the nervous symptoms. These symptoms are evidently of different nutritional origin than those described by Evans and Burr

(10). Additional evidence that Diet —A_9 contains adequate vitamin E has been supplied by Hogan and Harshaw (16), who found that hydrogenated cottonseed oil contained vitamin E.

TABLE 3.—The Relationship Between the Blood Picture and Nervous Symptoms of Rats on a Vitamin A-deficient Diet, Supplemented with Copper and Vitamin E

Rat No.	Diet variable*	Days on diet	Change in weight from maximum	Days on the diet before ophthalmia developed	Hemoglobin Grams per 100 cc.	Erythrocytes per c. mm.	Days on the diet before nervous symptoms developed
		No.	*Gm.*	*No.*		*No.*	*No.*
N 1♂	I	90	—22	51	16.3	9,730,000	69
N10♂	I	90	—24	48	17.0	11,700,000	62
N12♀	I	90	—24	51	17.0	10,000,000	76
N 2♂	II	84	— 6	42	17.0	10,900,000	56
N 4♀	II	46	—16 (Died)	35	.....	14,380,000 (4 days before death)	Showed no symptoms
N 9♂	II	67	—13	38	14.1	8,850,000	63
N 3♀	III	84	—21	56	16.1	8,850,000	70
N 5♀	III	90	—15	51	17.0	10,000,000	76
N 8♂	III	90	—28	50	15.3	9,500,000	69
N 6♂	IV	39	(Died 4 days af	ter ophthalm	ia appeare	d, records inco	mplete)
N11♀	IV	63	— 9	38	16.6	10,600,000	59
N 7♀	IV	84	— 8	38	15.6	8,720,000	77
		(This	rat was mo	ribund on the	42nd day and	was given	500 mg. butter fat)

*Diet variable I: —A_9 + 0.5 gm. irradiated yeast + 50 mg. wheat germ oil + 0.1% $CuSO_4$ in drinking water.
Diet variable II: —A_9 + 0.5 gm. irradiated yeast + 0.1% $CuSO_4$ in drinking water.
Diet variable III: —A_9 + 0.5 gm. irradiated yeast + 50 mg. wheat germ oil.
Diet variable IV: —A_9 + 0.5 gm. irradiated yeast.

THE TECHNIQUE OF NERVE EXAMINATION

Parts of the nervous systems from the animals in the preliminary studies were prepared for examination after the method of Marchi. Unfortunately, this technique proved to be unreliable in our hands; therefore, no microscopical diagnoses are reported.

The unreliability of the Marchi and osmic acid techniques has been discussed by Duncan (6) and Prickett (42). Most histological methods depend upon fixatives and stains which, by the development of artifacts in the tissue section, demonstrate the presence of certain groups or classes of chemical compounds. The success of such methods depends upon the constancy of the artifacts produced and the accuracy of their interpretation. Variations in time, concentration of stain, temperature, and the thickness of the section may produce variations in the results. This is particularly true where the interpretation depends upon differences in degree of intensity of the stain rather than on differential reactions.

Cramer and Lee (3), in studying the reaction of various lipids to polarized light, report true fats as being isotropic; whereas cholesterin, cholesterin esters, phosphatides, cerebrocides, and cholesterin fatty acid mixtures are anisotropic. The normal myelin sheath consists of phosphatides, cerebrocides, and sulphatides [Mathews (26)] and is almost entirely free from true fats (triglycerides).

The staining techniques commonly used for diagnosing myelin degeneration depend upon stains which, by the development of artifacts, demonstrate the presence of true fats. Hurst (21) pointed out that, when a myelinated nerve fiber degenerates, the myelin gradually changes in composition from a mixture of phospholipids to a mixture of triglycerides. Recognizing these facts it occurred to us that the polarizing microscope might be used for this purpose.

To test the accuracy of this assumption Wallerian degeneration was surgically produced as follows: Normal adult white rats of uniform age were anesthetized with ether and a one-half–centimeter segment of one sciatic nerve was removed. Eleven rats were so operated. These rats were subsequently sacrificed by exsanguination at intervals of 3, 6, 12, 18, and 24 hours and of 2, 3, 6, 9, 12, and 15 days. Within 5 minutes after death the second centimeter of the nerve distil to the place of sectioning was removed and placed in 10 per cent formalin. The second centimeter was used to eliminate the traumatic effects of the original operation on the nerve. Sciatic nerves from normal animals were used as controls.

After fixation for at least 24 hours the pieces of nerve were sectioned longitudinally with a freezing microtome at a thickness of 20 microns. The sections were floated onto slides and mounted in glycerine for examination.

Sections prepared in this manner were examined with a Leitz polarizing microscope[3] at a magnification of 200 diameters. When a longitudinal section of a myelinated nerve is observed in polarized light and between crossed Nicols, it will be found that if the stage of the microscope is rotated 360° the field will become alternately light and dark four times in each revolution. The places of greatest illumination are known as the points of greatest birefringence; the point of "extinction" refers to the position of the stage when the field is dark. Thus, one is able to distinguish the material in the field which is birefringent (i. e., becoming alternately light and dark) from any material which does not exhibit such alternation. Isotropic materials observed in polarized light and between crossed Nicols remain dark when the stage is rotated. Triglycerides are isotropic; therefore, if present in the myelin sheath, they can be seen as black bodies or areas, in contrast to the normal myelin which shows the property of birefringence. With the aid of this technique we were able to observe marked degenerative changes in the nerve which had been cut only 3 hours. This is many hours earlier than degeneration can be detected with certainty by other methods. The progress of the degeneration could be closely followed and was found to be almost complete in 15 days. Complete details of this technique of nerve examination, together with plates illustrating the progress of degeneration, will appear in the near future in the Anatomical Record.

The microscopic appearance of the nerves from animals on vitamin A-deficient diets is essentially the same as that of nerves from the operated animals. As a further check we were able to demonstrate that what appeared as relative intensity of color when stained with Sudan III and Scharlach R was strikingly shown by the loss of birefringence when examined in polarized light.

Figure II is a photomicrograph of a section of the sciatic nerve from the rat shown in Figure I. This photomicrograph shows the extensive myelin degeneration associated with avitaminosis A.

[3]We are indebted to the Department of Dental Research of the College of Dentistry, The Ohio State University, for the polarizing microscope and photographic equipment used in this study.

Figures IVa and IVb show the appearance of a normal sciatic nerve photographed with polarized light (IVa) and between crossed Nicols (IVb). (A section observed in polarized light appears identical with one in ordinary light except that the illumination is less intense in polarized light.) Figures Va and Vb show the appearance of a degenerating sciatic nerve from a rat on a vitamin A-deficient diet photographed with polarized light (Va) and between crossed Nicols (Vb).

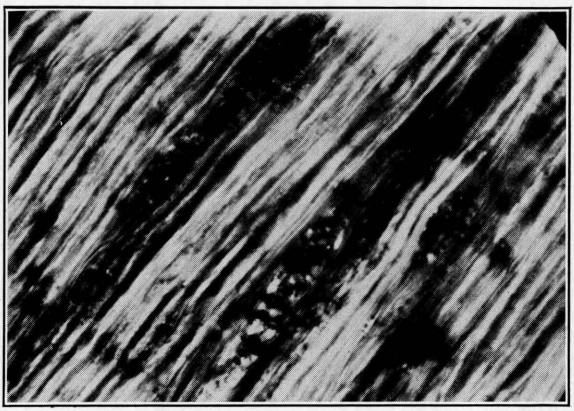

Fig. II. A photomicrograph of a longitudinal section of the sciatic nerve from rat C701 taken with polarized light and between crossed Nicols at the point of greatest birefringence. Note the swollen fibers and the large amount of isotropic material.

After careful checking of our technique and acquisition of skill through experience we were confident of accurate diagnoses. We believe that this is a highly satisfactory method of diagnosing the degenerative changes in myelinated nerves produced by vitamin A-deficient diets. It has the advantages of being simple and rapid and does not depend upon the development of artifacts.

NERVE DEGENERATION IN AVITAMINOSIS A

With a technique at hand which we felt could be relied upon, we were ready to continue the investigation of the relationship of nerve degeneration to vitamin A deficiency.

If this nervous disorder which we observed is caused by a lack of vitamin A in the diet, it should be possible to prevent it by including in the ration a small amount of material high in vitamin A activity. Since it has been shown that carotene can be substituted for vitamin A [Euler et al. (9), Moore (38), and Goldblatt and Barnett (13)] and can now be obtained in relatively pure crystals, it was selected for this study.

The rats used in this study were weaned when 24 days of age and placed in individual cages. The feeding of carotene was begun the same day the animal was weaned.

The carotene was fed in a cottonseed oil (Wesson Oil) solution at one and two gamma levels. The rats receiving no carotene received an equivalent

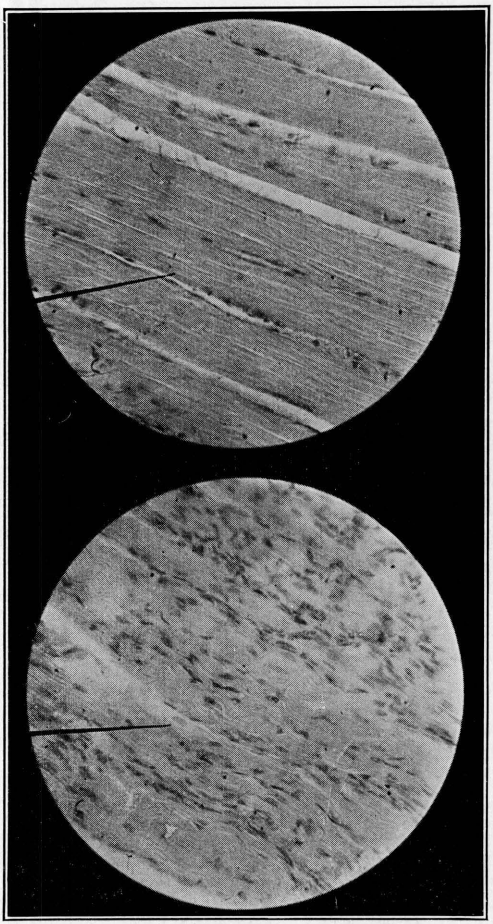

Fig. IIIa

Fig. IIIb

Fig. IIIa.—A photomicrograph of a normal biceps femoris of the white rat.

Fig. IIIb.—A photomicrograph of a biceps femoris of a rat showing the symptoms of nerve degeneration. Note the shrunken appearance of the fibers and the large number of fibroblasts.

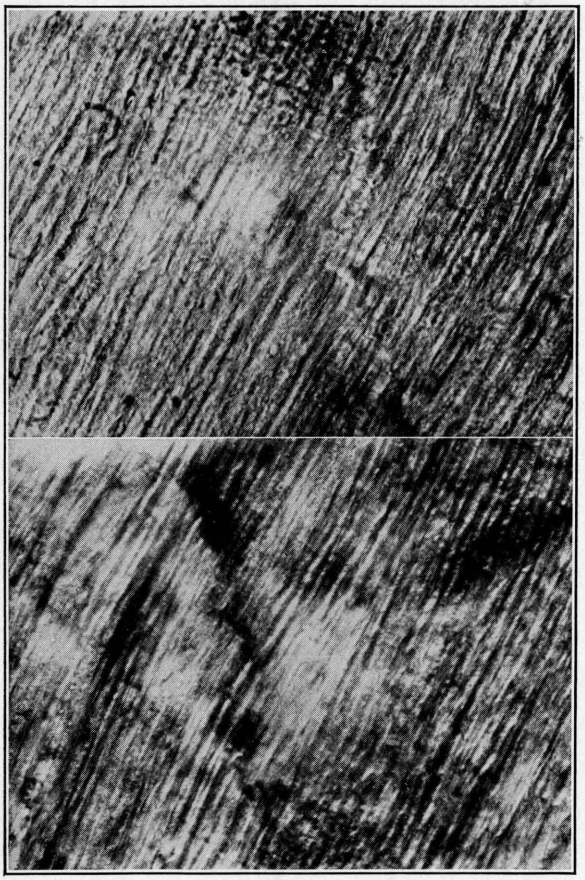

Fig. IVa

Fig. IVb

Fig. IVa.—A photomicrograph of a normal sciatic nerve taken with polarized light but not between crossed Nicols.

Fig. IVb.—A photomicrograph of the same field as Figure IVa taken with polarized light and between crossed Nicols at the point of greatest birefringence. Note the size of the fibers and the absence of vesicular areas of isotropic material.

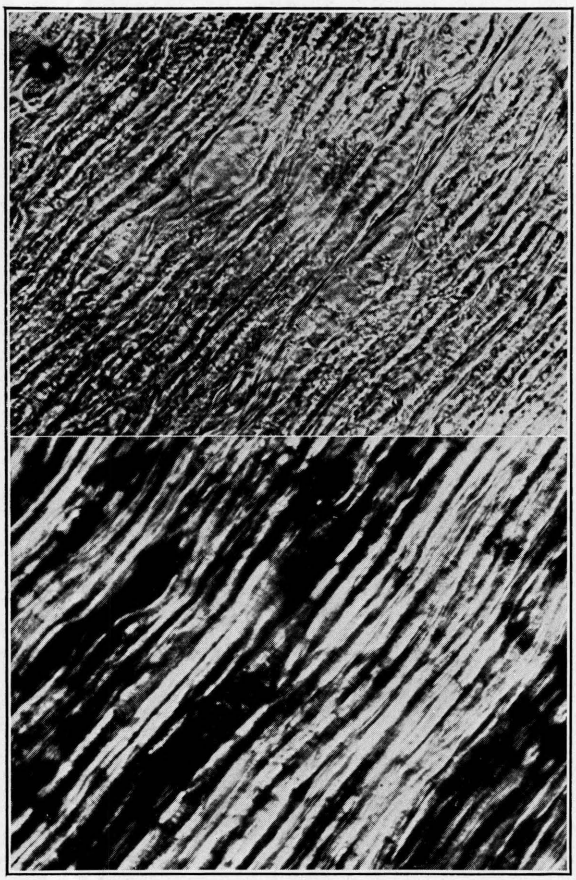

Fig. Va

Fig. Vb

Fig. Va.—A photomicrograph of a degenerating nerve taken with polarized light but not between crossed Nicols.

Fig. Vb.—A photomicrograph of the same field as Figure Va taken with polarized light and between crossed Nicols at the point of greatest birefringence. Note the swollen fibers and the vesicular areas of isotropic material.

amount of the oil. The animals in this study were further subdivided to test a basal ration containing dextrin and lard (—A_{10}) against one containing corn starch and Crisco (—A_9).

The results of this study are presented in Table 4. It will be noted that the animals receiving one and two gamma levels of carotene daily were protected against symptoms of avitaminosis A, including nervous symptoms.

TABLE 4.—The Influence of Carotene on Nerve Degeneration in Rats on Diets Otherwise Deficient in Vitamin A

(Rats killed at the end of 49 days, except Nos. N20, N22, N23)

Rat No.	Diet variable	Change of weight from maximum	Symptoms (duration before death or killing)	Microscopic diagnosis
Basal diet—A_{10} + 0.5 gm. irradiated yeast daily				
N 13 ♀	None	—16 gm.	Ophthalmia 16 days Incoordination 8 days	Extensive degeneration in sciatic nerve and spinal cord
N 14 ♂	2γ carotene	Still gaining	None	No degeneration in sciatic or cord
N 15 ♀	2γ carotene	Still gaining	None	No degeneration in sciatic or cord
N 16 ♀	1γ carotene	Still gaining	None	No degeneration in sciatic or cord
N 17 ♀	None	—14 gm.	Ophthalmia 16 days Incoordination 8 days	Extensive degeneration in sciatic, femoral, and cord
Basal diet—A_9 + 0.5 gm. irradiated yeast daily				
N 18 ♂	None	—21 gm.	Ophthalmia 16 days Incoordination 8 days	Scattered degeneration in cord Extensive degeneration in sciatic
N 19 ♀	None	—16	Ophthalmia 8 days No incoordination	Scattered degeneration in spinal cord
N 20 ♂	None	(Died, records	incomplete)	
N 21 ♂	None	(This rat was	moribund on 42nd day a	nd was given 0.5 gm. cod-liver oil)
N 22 ♂	1γ carotene	(These were c	None arried on 8 weeks longer)	No degeneration in sciatic or cord
N 23 ♀	2γ carotene		None	
N 24 ♀	1γ carotene	Still gaining	None	No degeneration in sciatic or cord

A microscopic examination of parts of the nervous system after the method just described showed degenerative lesions in the case of the animals receiving no carotene. The parts of the nervous system from the rats receiving carotene were normal. Rat N19 in this group showed no signs of nervous disorders, and yet an examination of the spinal cord revealed scattered degeneration in the myelinated fibers. Femoral and sciatic nerves were not studied. This observation indicated that nerve degeneration must precede the external symptoms of degeneration and possibly some of the other symptoms of the vitamin A deficiency disease as well.

To determine the accuracy of this assumption a fourth series of rats was placed on a vitamin A-deficient diet (—A_9). Rats of similar breeding on the same diet were known to have a high incidence of ophthalmia on the thirty-fifth day of depletion. For this reason it was decided that beginning about a week earlier (on the twenty-eighth day) some of the animals would be sacrificed for neurological examination and others thereafter at intervals until definite nervous symptoms were noted. Since none of the remaining rats

showed signs of ophthalmia earlier than the thirty-fourth day, it is safe to assume that the first animals were killed about 6 days before ophthalmia would have appeared.

Table 5 shows a summary of the data obtained on this group. Only the femoral and sciatic nerves were studied. A word of explanation concerning the arbitrary standard of diagnosis may be necessary to clarify the records under the heading "Microscopic Diagnosis". The half plus sign indicates changes from the normal occurring before the appearance of isotropic material in the myelin sheath. These changes consist of a swollen, slightly lobulated and irregular appearance of the myelin sheath and a swelling of the axis cylinder. A single plus sign indicates the actual presence of isotropic material in the myelin sheath. Two, three, and four plus signs are used to indicate increasing amounts of isotropic material, together with the involvement of an increasing number of fibers. Figure 6 illustrates this progress of degeneration in the sciatic nerve and presents an example of the standard of diagnosis.

TABLE 5.—The Progress of Nerve Degeneration in Avitaminosis A

[Basal diet —A_9+0.5 gram of yeast+2.6 units of vitamin D (viosterol) daily]

Rat No.	Days on deficient diet	Duration of ophthalmia	Change in weight from maximum	Microscopic diagnosis	
				Femoral	Sciatic
C 802 ♀....	28	No ophthalmia	Still gaining	Normal	⊥
C 805 ♀....	28	No ophthalmia	Still gaining	Normal	⊥
C 804 ♂....	31	No ophthalmia	Still gaining	Normal to ⊥	Normal to ⊥
C 807 ♀....	31	No ophthalmia	Still gaining	Normal	Normal to ⊥
C 803 ♀....	34	Incipient ophthalmia	Still gaining	+	+
C 806 ♀....	34	Incipient ophthalmia	Still gaining	+	+
C 812 ♂....	34	Incipient ophthalmia	Still gaining	+	+
C 813 ♀....	34	Incipient ophthalmia	Still gaining	+	+
C 668 ♀....	39	3 days	Still gaining	+	+
C 673 ♀....	39	3 days	Still gaining	+	+
C 667 ♂....	43	7 days	— 5 gm.	+	++
C 675 ♂....	43	7 days	— 9 gm.	++	++
C 671 ♂....	50	7 days	—12 gm.	+++	+
C 670 ♂....	56	10 days	—34 gm.	+++ (Wobbly gait in	+++ rear limbs)
C 672 ♂....	56	10 days	—16 gm.	+++ (Impaired use of	+++ right foot)
C 674 ♂....	60	20 days	—25 gm.	++++ (Impaired use of	++++ left foot)
C 666 ♀....	72	26 days	—21 gm.	+++ (Wobbly gait, p	++++ oor coordination)

The diagnoses were made in a purely objective manner, the operator knowing nothing of the condition of the animal from which the material for examination was obtained. Nerves from a rat of approximately the same age which had a complete diet were used for reference.

The data presented in Table 5 show that changes take place in the nerves prior to the appearance of ophthalmia. Degenerative changes were noted in both the femoral and sciatic nerves of rats showing incipient ophthalmia. The degeneration progresses as the animal is continued on the deficient diet and is extensive before external symptoms are noted.

We had previously noted that external symptoms indicative of nerve degeneration did not occur among the groups of rats fed a high level of vitamin A supplement. Therefore, it was thought advisable to study the nerves from groups of rats receiving high and low levels of vitamin A supplements after depletion.

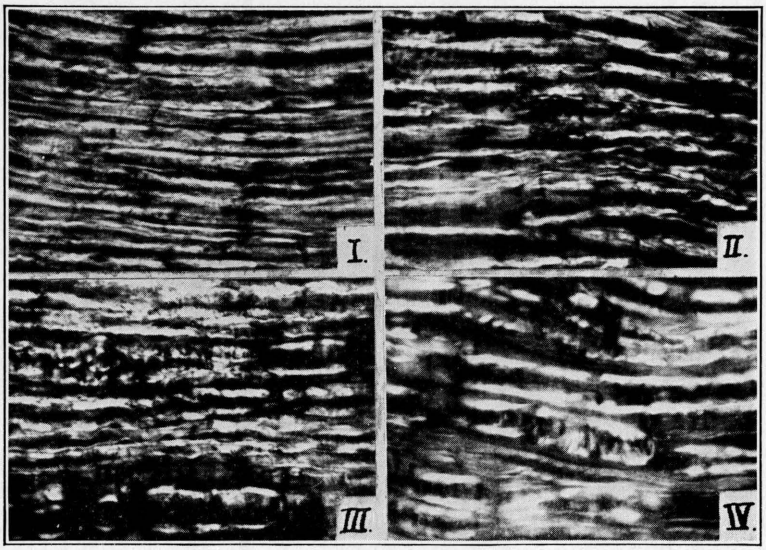

Fig. VI.—These photomicrographs show the progress of nerve degeneration in avitaminosis A and illustrate the arbitrary standard of diagnosis: I, + degeneration; II, ++ degeneration; III, +++ degeneration; and IV, ++++ degeneration. For comparison with a normal nerve refer to Fig. IVb.

The litters of rats set aside for this purpose were weaned when 24 days of age and placed on Diet —A_{11} (a diet containing both Crisco and lard and starch and dextrin). The basal diet was supplemented with 0.5 gram of irradiated yeast fed separately each day. When the rats were depleted of vitamin A, as shown by cessation of growth and incipient ophthalmia, the vitamin A supplement was fed with the yeast. The original plan was to feed butterfat at 30 and 90 mg. levels, cod-liver oil at 1.0 and 3.0 mg. levels, and carotene at 0.4 and 1.2 gamma levels. However, at the end of 3 weeks the rats receiving 0.4 gamma of carotene were in an extremely weak condition, and the dosage was increased to 0.8 gamma for the remaining 5 weeks. This increase produced a rapid acceleration of growth during the remaining 5 weeks. Such a phenomenal response was to be expected since Goldblatt and Barnett (13) have shown that 0.5 gamma of carotene will produce unit growth (3 grams a week over an 8-week period) in rats on a vitamin A-free diet. A summary of the results of this study is presented in Table 6.

A histological study of the femoral and sciatic nerves of this group produced evidence that nerve degeneration progresses when the diet is inadequately supplemented. The eye condition of the animals on the low level of supplement feeding improved for a short time after the supplement was added to the ration but became worse again in all the animals except those receiving 0.8 gamma of carotene. At the end of the 8-week period the rats receiving 0.8 gamma of carotene were in good physical condition, except for the partial paralysis of the rear limbs. When the higher levels of supplements were fed, no nervous symptoms developed and a histological examination of the femoral

and sciatic nerves showed that the degeneration had been arrested. If the microscopic diagnoses of the animals in this group are compared with those in Group V, it will be seen that the condition of the nerves after 8 weeks of high vitamin A feeding is approximately the same as that of animals showing incipient ophthalmia.

TABLE 6.—The Influence of High and Low Levels of Vitamin A Supplements on Nerve Degeneration in Rats Depleted of Vitamin A

Unless otherwise designated all rats were killed after 8 weeks of supplementary feeding. (Basal Diet —A_{11} + 0.5 gm. irradiated yeast)

Rat No.	Supplement	Change in weight during supplementary feeding	Duration of nervous symptoms before killing or death	Microscopic diagnosis (Femoral and sciatic nerves)
C688♀	30 mg. B. F.	— 5* (42 days)	7 days	+++
C676♂	30 mg. B. F.	+20* (29 days)	8 days	++++
C694♀	30 mg. B. F.	−16* (43 days)	21 days	+++
C682♀	30 mg. B. F.	— 2	21 days	+++
C679♀	90 mg. B. F.	+56	No symptoms	+
C685♂	90 mg. B. F.	+56	No symptoms	⊥
C690♀	1.0 mg. C.L.O.	— 6* (46 days)	14 days	+++
C677♂	1.0 mg. C.L.O.	+16	21 days	+++
C695♂	1.0 mg. C.L.O.	−10* (41 days)	13 days	+++
C686♀	1.0 mg. C.L.O.	+28	28 days	++
C680♀	3.0 mg. C.L.O.	+50	No symptoms	+
C683♂	3.0 mg. C.L.O.	+66	No symptoms	⊥
C692♀	0.4γ carotene	+34	14 days	++
C678♀	for 3 weeks; 0.8	+51	28 days	+++
C696♀	γ carotene for	+25	42 days	+++
C684♂	5 weeks.	+54	Kept for demonstration	
C681♂	1.2γ carotene	+52	No symptoms	++
C687♀	1.2γ carotene	+75	No symptoms	⊥
C699♂	1.2γ carotene	+64	Kept for demonstration	

*Rat died before end of the experiment.

Careful record of the incidence of nervous symptoms among rats used for vitamin A assays has been kept. The data presented in Table 7 show the incidence of nervous symptoms among rats used in assaying the same samples of butterfat. The butterfat was fed at 30, 45, and 60 milligram levels. It so happened that 45 milligrams constituted approximately a unit dose. Less than half of the rats receiving 30 milligrams of the butterfat survived the 8 weeks' curative period. However, life was sufficiently prolonged to allow the development of a high incidence of nervous symptoms. The symptoms were extreme in many of these cases. At the 45-milligram level the incidence was not nearly so great, and there were few extreme cases. The symptom most frequently encountered in this group was a noticeable abduction of one rear limb. No symptoms of nervous disorders were noted among the rats receiving butterfat at the 60-milligram level.

Femoral and sciatic nerves from 21 of the rats receiving 30 milligrams of butterfat were examined. Nine showed +++ and 12 ++++ degeneration. No microscopic diagnoses were made in the case of animals receiving 45 and 60 milligram dosages of butterfat.

TABLE 7.—The Occurrence of Nervous Symptoms in Rats Receiving a Vitamin A-deficient Diet, Supplemented with Varying Levels of Butterfat after Cessation of Growth and Appearance of Ophthalmia

[Basal diet —A_9+0.5 gram of yeast+2.6 units of vitamin D (viosterol) daily]

Number of rats	Level of butterfat supplement	Per cent of rats showing nervous symptoms	Microscopical diagnosis
20	60 mg. daily	0	No microscopic diagnosis made
28	45 mg. daily	42.8	No microscopic diagnosis made
48	30 mg. daily	97.9	The femoral and sciatic nerves from 21 of these rats were examined microscopically; 9 showed + + + degeneration and 12 showed + + + + degeneration

DISCUSSION

The data obtained in this study provide evidence that vitamin A is necessary for maintaining the nervous system intact. Mellanby (34) has pointed out the necessity of vitamin A to protect the nervous system against toxic substances in a cereal diet. Toxic properties were attributed to the germ of cereals and ergot; however, nerve lesions were not absent when these materials were left out of the vitamin A-deficient diet. He was probably dealing with at least two etiological factors; first, the lack of vitamin A, and, secondly, the toxic material which he added to the diet. The convulsive symptoms which he described lend weight to this hypothesis. Ergot alone is sufficient to cause severe nervous symptoms, including convulsions and tetany.

Zimmerman (51) has shown that the absence of vitamin A from the diet is sufficient in itself to cause nerve degeneration in rats. While there are some disturbing differences in the description of external symptoms, the similarity of the dietary conditions of production indicates that we are dealing with the same pathological condition as Zimmerman.

The diets we used contained all the known dietary essentials except vitamin A. These diets were not anemia producing. A 25 per cent increase in the yeast supplement over what was used in the preliminary studies did not prevent or retard the development of the symptoms, indicating that the B complex was not a causative factor. The addition of small amounts of material high in vitamin A activity was sufficient to prevent all the symptoms of a deficiency disease, including nervous symptoms.

Mellanby's prevention of nerve lesions by vitamin A, while somewhat similar lesions and symptoms were produced by Stockman and Johnston (47) with diets containing adequate vitamin A, indicates that these workers were dealing with different causative factors. Both, however, attributed the degeneration to toxic substances contained in cereals. Mellanby's toxic principle is an unknown substance found in the germ of cereals or in ergot. The diet we employed contained neither of these materials. The toxic substance of Stockman and Johnston is an acidic compound of cereals found concentrated in the residue after the starch and oil have been extracted. This compound can be removed by water extraction. In the commercial preparation of corn starch the starch is washed and allowed to settle out of water. This process would remove most of this toxic compound were there any present in the starch portion of the grain.

The striking parallelism between the progress of nerve degeneration and the avitaminosis A syndrome is suggestive of the same etiology. Zimmerman was unable to demonstrate that nerve degeneration preceded the clinical symptoms by any appreciable period. He also reports recovery in all his animals except one after a vitamin A supplement was added to the diet.

Aberle (1) has shown that the earliest symptoms of nervous disorders are always preceded by cornified vaginal smears and frequently by ophthalmia and cessation of growth.

We were able to demonstrate slight changes in the sciatic and femoral nerves before the earliest signs of the vitamin A-deficiency disease, and degenerative changes were always noted in the nerves of animals showing incipient ophthalmia. It is possible that we were able to do this because we employed a more sensitive technique than Zimmerman. Early degenerative lesions of the order noted at the time of incipient ophthalmia were unhealed in rats which had received adequate vitamin A for a period of 8 weeks after ophthalmia appeared. The muscle atrophy which is probably secondary to the degeneration of trophic fibers seems to be irreparable. We have put several of these animals on our stock diet for periods as long as 4 months with no complete recoveries. While no histological studies were made by Krauss and Hayden (25), the similarity of the conditions of production and the response to treatment indicate that the condition which we have studied is the same as they described. The animals grow and appear to be in excellent physical condition except for the affected limb or limbs.

There are several possible reasons why the symptoms of nervous disorders have not been described by other workers employing similar vitamin A-deficient diets. Inquiry among a number of workers in various laboratories reveals that these nervous disorders have been observed but have been given no particular attention. We have noted in connection with other studies conducted in this laboratory that the development of these symptoms is delayed in animals that have a large body store of vitamin A at the beginning of the depletion period. The stock diet employed may be important in this respect. To obtain a high incidence of the symptoms, less than a unit dosage of vitamin A must be administered after depletion. If a unit dosage or excess of a unit dosage is administered, the degeneration is arrested or retarded to such an extent that many of the animals do not show clinical symptoms.

At times, when respiratory infections are prevalent among rats on vitamin A-deficient diets, death from bronchopneumonia usually occurs before nervous symptoms are noted in negative controls. Our laboratory has been unusually free from respiratory infections during the past year and we were able to demonstrate the symptoms and lesions in many negative controls.

The immediate cause of epithelial changes occurring in vitamin A avitaminosis has never been adequately explained. It is possible that early degenerative changes in the nerves are responsible for the metaplasia of epithelial tissues. Another possible explanation of the tissue changes lies in the fact that all the tissues involved are high in phospholipid content. That the normal phospholipids of the myelin sheath undergo chemical transformation in the degenerative process is demonstrated in this study. Changes in the phospholipids of the epithelial cells resulting in changes in the cell permeability may be in part responsible for the metaplasia. Outside of the demonstration of small fat droplets in the cytoplasm of cells involved in these tissue changes by Mori (39), little direct evidence has been produced supporting this theory.

However, Monaghan and Schmitt (36) suggested the probability of vitamin A being a regulator of phospholipid metabolism, since they were able to show that vitamin A and carotene would prevent the oxidation of unsaturated fatty acids in vitro.

In nerve degeneration the myelin changes from a mixture of phospholipids to a mixture of triglycerides (21). The nature of this change, together with the lack of interfering compounds, makes possible the use of the polarizing microscope in determining the presence and approximate extent of degeneration in formalin-fixed frozen sections. This technique is simple and rapid and, we believe, more accurate than the common staining techniques.

CONCLUSIONS

A series of studies involving the histological examination of parts of the nervous system of rats on vitamin A-deficient diets produced data pointing to the following conclusions:

1. Degeneration of the myelin sheath of peripheral nerves is a part of the syndrome of tissue changes in avitaminosis A.

2. The degeneration can be prevented by supplementing the diet with one gamma of carotene daily.

3. The variations in the vitamin A-deficient basal diets employed produced no significant difference in the results.

4. The degeneration occurs at about the same time as ophthalmia. It progresses when the animal receives no vitamin A supplement after ophthalmia appears and is extensive before external symptoms of paralysis are noted.

5. When the deficient diet is inadequately supplemented after depletion, the degeneration progresses and external symptoms are noted. Adequate dosages of vitamin A supplements arrest the degeneration but do not relieve the external symptoms, once they have appeared.

6. The examination of formalin-fixed, frozen sections with a polarizing microscope is a satisfactory method for diagnosing degenerative changes in peripheral nerves of rats suffering from a deficiency of vitamin A.

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